March 4, 2003

Timothy Adams, Ph.D.
Technical Contact
The Flavor and Fragrance High Production Volume Consortia
The Terpene Consortia
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Dear Dr. Adams:

The Office of Pollution Prevention and Toxics is transmitting EPA's comments on the robust summaries and test plan for Estragole posted on the ChemRTK HPV Challenge Program Web site on November 4, 2002. I commend The Flavor and Fragrance High Production Volume Consortia's Terpene Consortia for their commitment to the HPV Challenge Program.

EPA reviews test plans and robust summaries to determine whether the reported data and test plans will provide the data necessary to adequately characterize each SIDS endpoint. On its Challenge Web site, EPA has provided guidance for determining the adequacy of data and preparing test plans used to prioritize chemicals for further work.

EPA will post this letter and the enclosed Comments on the HPV Challenge Web site within the next few days. As noted in the comments, we ask that The Flavor and Fragrance High Production Volume Consortia's Terpene Consortia advise the Agency, within 60 days of this posting on the Web site, of any modifications to its submission.

If you have any questions about this response, please contact Richard Hefter, Chief of the HPV Chemicals Branch, at 202-564-7649. Submit questions about the HPV Challenge Program through the "Contact Us" link on the HPV Challenge Program Web site pages or through the TSCA Assistance Information Service (TSCA Hotline) at (202) 554-1404. The TSCA Hotline can also be reached by e-mail at tsca-hotline@epa.gov.

I thank you for your submission and look forward to your continued participation in the HPV Challenge Program.

Sincerely,

-*S*-

Oscar Hernandez, Director
Risk Assessment Division

Enclosure

cc: C. Auer
A. Abramson
W. Penberthy
M. E. Weber

EPA Comments on Chemical RTK HPV Challenge Submission:
Estragole

Summary of EPA Comments

The sponsor, the Terpene Consortium of the Flavor and Fragrance High Production Volume Consortia (FFHPVC), submitted a test plan and robust summaries to EPA for estragole (p-allylanisole, CAS No. 140-67-0) dated October 21, 2002. EPA posted the submission on the ChemRTK HPV Challenge Web site on November 4, 2002.

EPA has reviewed this submission and has reached the following conclusions:

1. Analog Justification. In some sections of the test plan, the analogs selected and justifications provided were insufficient to support the use of surrogate data to satisfy an endpoint. For health effects, methyl eugenol is a reasonable analog whereas *trans*-anethole does not appear to be an appropriate analog.

2. Physicochemical Properties. The data provided by the submitter for boiling point, vapor pressure, water solubility, and partition coefficient are adequate for the purposes of the HPV Challenge Program. The submitter needs to provide measured melting point data for this chemical.

3. Environmental Fate. The data provided for photodegradation and fugacity are adequate for the purposes of the HPV Challenge Program. EPA agrees that biodegradation testing should be conducted for this chemical. The submitter needs to address some deficiencies in the robust summaries.

4. Health Effects. Adequate data are available for the acute toxicity endpoint for the purposes of the HPV Challenge Program. EPA reserves judgement on the adequacy of the genetic toxicity and repeated-dose toxicity endpoints pending submission of additional critical information. EPA believes that *trans*-anethole is not an appropriate analog for estragole given its different metabolic profile and thus does not support the reproduction and developmental toxicity endpoints for estragole. EPA recommends that the submitter provide data from an appropriate analog or conduct a combined reproduction/developmental toxicity screening test on estragole.

5. Ecological Effects. EPA agrees with the test plan for these endpoints. However, the submitter needs to address deficiencies in the robust summaries.

EPA requests that the submitter advise the Agency within 60 days of any modifications to its submission.

EPA Comments on the Estragole Challenge Submission

Test Plan

Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility).

The data provided by the submitter for boiling point, vapor pressure, water solubility, and partition coefficient are adequate for the purposes of the HPV Challenge Program.

Melting point. The submitter provided calculated data for this endpoint from the EPIWIN program. EPIWIN has sometimes provided melting point data that do not match experimental data, and therefore should not be used as the only source of melting point information. Furthermore, the use of estimated values introduces uncertainties that then become magnified in modeling applications. The submitter

needs to provide measured melting point information following OECD Guideline 102, or provide data from a reliable literature source.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity).

The data provided by the submitter for photodegradation are adequate for the purposes of the HPV Challenge Program.

Stability in water. While the test plan correctly states that estragole cannot hydrolyze, the submitter also needs to provide a brief explanation in robust summary format.

Biodegradation. EPA agrees with the submitter's recommendation that biodegradation testing should be conducted for this chemical. The submitter needs to provide ready biodegradation data following OECD Guideline 301.

Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity).

Acute Toxicity. The acute oral toxicity data are adequate for the purposes of the HPV Challenge Program on a weight-of-evidence basis.

Genetic Toxicity. EPA reserves judgement on the adequacy of gene mutation and chromosomal aberration endpoints pending submission of the additional critical information for the robust summaries.

Repeated-Dose Toxicity. EPA reserves judgement on this endpoint for the following reasons:

> (1) All the submitted studies assessed carcinogenicity as an endpoint. Except for the methyl eugenol NTP cancer bioassay range-finding study, these studies were not complete enough to satisfy the repeated-dose endpoint requirements.
>
> (2) The 14-week repeated-dose studies performed as range-finding studies for the methyl eugenol cancer studies were not presented or summarized. The submitter needs to provide summaries for the 14-week methyl eugenol studies. This information may be important because of the testicular effects observed in mice (NTP, 2000).
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> (3) NTP recently completed a 90-day study with estragole in rats and mice. According to the NTP website (http://ntp-server.niehs.nih.gov/), a 90-day study with estragole was started in September of 2001. At this time (March, 2003), there is no indication what the results were or when a draft report will be available. EPA believes this information to be critical as it addresses the toxicity of the sponsored chemical. The submitter needs to check on the status of this study.

Reproduction and Developmental Toxicity. The submitter has used *trans*-anethole data to address the reproductive and developmental toxicity endpoints. Although *trans*-anethole is structurally similar to estragole, its metabolic pathway and thus its toxicity may be significantly different at higher dose levels. Submitted Information for estragole and additional information in a separate HPV submission for anethole (posted on the ChemRTK Web site December 4, 2002) describe O-demethylation as the predominant pathway at lower intake levels for both substances, but at higher levels (greater than 10 mg/kg bw), estragole (and methyl eugenol) utilize a 1'-hydroxylation pathway producing reactive intermediates that have been associated with toxicity. However, in the case of *trans*-anethole, when the O-demethylation pathway is saturated, omega-oxidation occurs producing end products similar to those seen with estragole but without the formation of the reactive intermediate. Also, both estragole and *trans*-anethole can form epoxides; however, the epoxidation pathway, as presented, is a minor metabolic route. Since the

formation of a reactive intermediate is an important difference in the metabolism of estragole, *trans*-anethole is not an appropriate surrogate for estragole, especially at higher doses.

Other studies submitted for the reproductive and developmental toxicity endpoints used a mixture referred to as oil of nutmeg or FDA 71-28, defined as a mixture of 10-20% p-allylalkoxybenzene derivatives (myristicin, elemicin, safrole and methyl eugenol) and 80 to 90% bicyclic terpene hydrocarbons. Although methyl eugenol is a reasonable surrogate for estragole, the amount present in this mixture is very small and suggests that the mixture itself may not be a good analog for estragole. No comparison of the metabolic pathways for the other p-allylalkoxybenzene derivatives–or the bicyclic terpene–components of the mixture has been supplied and therefore the information provided does not address the reproductive/developmental toxicity endpoint for estragole.

EPA recommends that the submitter provide data from a more appropriate analog or conduct a combined reproduction/developmental toxicity screening test (such as OECD 421) on estragole.

Ecological Effects (fish, invertebrates, and algae).

The test plan for these endpoints is adequate for the purposes of the HPV Challenge Program. The submitter needs to provide missing study details in the robust summaries.

Specific Comments on the Robust Summaries

Generic comments

The submitter should consult EPA guidance documents for the preparation of robust summaries (http://www.epa.gov/opptintr/chemrtk/guidocs.htm).

Summaries should list the substance purity or explicitly state if that information was not reported.

In the robust summaries for analogs, it would be preferable to use the analog name as the Substance Name, with analog status indicated in parentheses, and show the CAS number of the analog. For example:

Substance Name	Safrole (analog for estragole)
CAS No.	94-59-7

For test mixtures, each robust summary needs to include all available compositional information. For example, test material FDA 21-78 was only completely defined on page 29 of the test plan; none of the robust summaries mentioned that it contained 80-90% bicyclic terpenes (not identified as estragole analogs).

In some cases, the submitter did not use the term NOAEL/NOEL correctly. If the lowest dose was a LOAEL, the study did not have a NOAEL since these terms refer to 'observed', i.e., tested levels; in this situation, the correct NOAEL/NOEL field is 'undetermined' or 'none.'

A positive control is required for genotoxicity assays. Negative genotoxicity data are not valid if the summaries do not report the positive controls.

Environmental Fate (photodegradation, stability in water, biodegradation, fugacity).

Transport and distribution (fugacity). The data provided by the submitter for transport and distribution (fugacity) are adequate for the purposes of the HPV Challenge Program. However, the submitter needs to incorporate in the robust summary the actual values of the input parameters used in the model.

Health Effects

Acute Toxicity. The Jenner et al. (1964) study summaries should provide the doses used and the length of the observation period, and whether there were any gross necropsy findings.

Repeated-Dose Toxicity. 12-month cancer study with estragole. A robust summary for a 12-month carcinogenesis assay (Miller et al., 1983) in mice exposed to estragole or its metabolite, 1-hydroxyestragole, in the diet provided some information on systemic toxicity (noncancer effects), but was incomplete. Omissions included the purity of the test material, the group sizes, the sizes of body weight gain reductions, time-weighted averages for the doses (modified during the test), and information about organ weights or histopathology of organs besides the liver.

Genetic Toxicity. Ames assays. All the summaries of mutation assays in *Salmonella typhimurium* need the following: information on positive controls (except for To et al., 1982), the cytotoxic concentration, the number of replicates, and the statistical methods or the criteria for determining levels of significance.

In vitro chromosomal aberration study. A robust summary for a negative chromosomal aberration assay in cultured rat V79 cells omitted information on the use of positive controls, the concentrations administered, the criteria for positive results, and the numbers of cells examined.

Ecological Effects

Fish. The robust summary submitted for a study with *trans*-anethole did not indicate the number of fish tested per concentration, control use and response data, and the statistical methods used.

Invertebrates. The two robust summaries submitted for studies with estragon oil (70-88% estragole) and *trans*-anethole did not indicate one or more of the following study details: the number of organisms tested per concentration, control response data, signs of toxicity/mortality data, statistical methods used, and/or the test system used (i.e., static vs. renewal or flow-through).

Algae. The robust summary submitted for a study with *trans*-anethole reported an 96-hour IC_{50} value rather than a 72-hour LC_{50} or NOEC value. Missing study details included control response data and water chemistry measurements.

Followup Activity

EPA requests that the submitter advise the Agency within 60 days of any modifications to its submission.

References

Smith et al. (2002). Safety assessment of allylalkoxybenzene derivatives used as flavouring substances–methyl eugenol and estragole. Food Chem. Toxicol., July, vol. 40(7): 851-70. (This report is listed as an unpublished report in the robust summary.)

National Toxicology Program (NTP), 2000. Toxicology and carcinogenesis studies of methyl eugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950.